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EFFECTS OF CYCLIC AMP, OUABAIN AND FUROSEMIDE ON ION TRANSPORT IN ISOLATED CANINE GASTRIC MUCOSA

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SUMMARY

1. Cyclic AMP (10 mM), present in the serosal solution of isolated dog gastric mucosa, increased potential difference (p.d.), short-circuit current (I_{SC}), net flux of Na^+ from the mucosal to serosal side, and the unidirectional flux of K^+ from the mucosal to serosal side. Cyclic AMP did not stimulate H^+ or Cl^- secretion.

2. Dibutyryl cyclic AMP (DBcAMP, 1 mM) or theophylline (2 mM), present in the serosal solution, stimulated H^+ and Cl^- secretion, decreased p.d., I_{SC} and electrical resistance. These compounds had no effect on Na^+ transport. The stimulatory effect of DBcAMP on H^+ secretion was still present after pretreatment with cimetidine or atropine.

3. Ouabain abolished both the p.d. and I_{SC} of the histamine-stimulated gastric mucosa. The mucosal to serosal flux of Na^+ and the serosal to mucosal flux of Cl^- were significantly decreased in the presence of ouabain. Ouabain caused an increase in the serosal to mucosal flux of K^+ and high concentrations caused a significant reduction in H^+ secretion.

4. Furosemide (10^{-4} M) decreased p.d., I_{SC} and net flux of Na^+. Higher concentrations inhibited the net flux of Cl^- from the serosal to mucosal side.

5. These results suggest that in isolated dog gastric mucosa, (1) both DBcAMP and theophylline may increase intracellular cyclic AMP to stimulate H^+ and Cl^- secretion, (2) cyclic AMP, outside the serosal membrane, stimulates active transport of Na^+; in contrast, ouabain inhibits this active process predominantly, (3) the selective action of furosemide on Na^+ transport indicates that Na^+ and Cl^- move via separate transport pathways across the serosal border.

INTRODUCTION

The basic mechanisms of ion transport (H^+, Cl^-, Na^+ and K^+) in mammalian gastric mucosa have been explored using a previously reported preparation (Kuo & Shanbour, 1978, 1979) of isolated canine gastric mucosa. The following agents were examined to dissect the mechanisms involved in H^+ secretion, as well as ion transport of Cl^-, Na^+ and K^+: cyclic AMP, a proposed intracellular mediator between secre-

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tagogues and H^+ secretion; theophylline, an inhibitor of phosphodiesterase (increases intracellular cyclic AMP); ouabain, an inhibitor of Na^+-K^+-ATPase; and furosemide, an inhibitor of Na^+ transport. The effects of these agents on gastric electrophysiological activity were also evaluated.

METHODS

Materials

Cyclic AMP (adenosine 3′:5′ cyclic monophosphoric acid), theophylline, DBcAMP (N^6, $O^{2'}$ dibutyryl adenosine 3′:5′ cyclic monophosphoric acid) and atropine were obtained from Sigma Chemical Company (St Louis, Mo.); ouabain was from Eli Lilly and Company (Indianopolis, Ind.); cimetidine was from Smith Kline and French Laboratories (Carolina, P.R.). Furosemide was a generous gift of Hoechst-Roussel Pharmaceuticals (Somerville, N.J.). All other reagents used were of analytical grade.

Radioactive ^{22}Na, ^{36}Cl and ^{42}K were obtained as aqueous solutions from New England Nuclear (Boston, Ma.).

Animals – Ringer solutions

Mongrel dogs, 10–15 kg in weight, were fasted for 48 hr and anaesthetized with chloralose-urethane. The preparation of the gastric mucosa, stripped of its serosal and external muscle layers, has been previously described (Kuo & Shanbour, 1978).

The serosal Ringer solution contained (mM) 135·0 Na^+, 5·0 K^+, 1·0 Ca^{2+}, 1·0 Mg^{2+}, 115·0 Cl^-, 25·0 HCO_3^-, 1·0 SO_4^{2-}, 1·0 HPO_4^{2-} and 25·0 glucose. The mucosal solution was an unbuffered solution containing (mM) 135·0 Na^+, 5·0 K^+, 1·0 Mg^{2+}, 115·0 Cl^-, 13·5 SO_4^{2-} and 39·5 mannitol. All solutions were gassed with 95% O_2–5% CO_2.

Flux measurements and voltage-clamp system

The procedure, which was used for bidirectional flux measurements under voltage-clamp conditions, has been previously described (Kuo & Shanbour, 1979). Briefly, the mucosae were short-circuited and the I_{SC} was iterrupted periodically to determine the spontaneous transmucosal p.d. with an automatic voltage-clamp system (Shanbour, 1974). The resistance was calculated as the ratio of open-circuit p.d. to I_{SC}. Bidirectional fluxes of Na^+, Cl^- or K^+ were determined by adding ^{22}Na, ^{36}Cl or ^{42}K ($\sim$ 3 μc) into opposite sides of paired mucosae. After radioisotopic equilibration (60 min for ^{22}Na and ^{36}Cl, and 100 min for ^{42}K), solutions from the unlabelled half-chambers were collected and replaced every 20 min with fresh and pre-warmed solutions. Unidirectional fluxes of Na^+, Cl^- or K^+ were determined in one direction from one chamber and in the opposite direction from the other chamber. Details of the counting procedures and calculations have been previously described (Kuo & Shanbour, 1979).

Measurement of secretory rate

The rate of gastric acid secretion was measured by the pH-stat method (Kuo & Shanbour, 1978). The radiometer of a Copenhagen pH stat (pH meter 28, titrator 11 and autoburette 11) was used. The pH of the mucosal solution was maintained at a constant value between 4·7 and 5·0 by the addition of standardized 0·02 or 0·005 N-NaOH.

Statistics

Results are expressed as means ± S.E. Differences were considered significant if the P value, calculated from the paired Student t test, was less than 0·05.

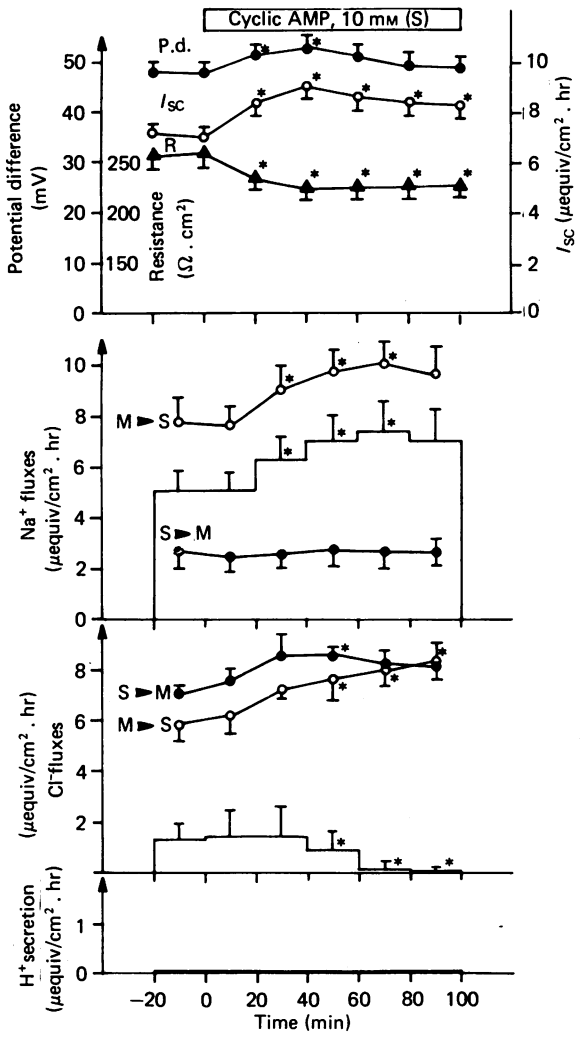

Fig. 1. Effects of cyclic AMP on electrical parameters (p.d. = potential difference, I_{SC} = short-circuit current, R = resistance), Na^+ fluxes (n = 5), Cl^- fluxes (n = 3) and H^+ secretion (n = 4) in isolated dog gastric mucosa. M → S, unidirectional flux from mucosal to serosal side. S → M, unidirectional flux from serosal to mucosal side. The histogram represents net flux.

* Significant difference from values at time zero ($P < 0{\cdot}05$, paired Student t test).

RESULTS

Effects of cyclic AMP

Fig. 1 illustrates the effects of cyclic AMP on Na^+ fluxes, Cl^- fluxes, H^+ secretion and electrical parameters in isolated dog gastric mucosae. In the control period (−20 min to zero time), potential difference, I_{SC} and R were stable; there was no spontaneous H^+ secretion; net flux of Cl^- from the serosal to mucosal side was

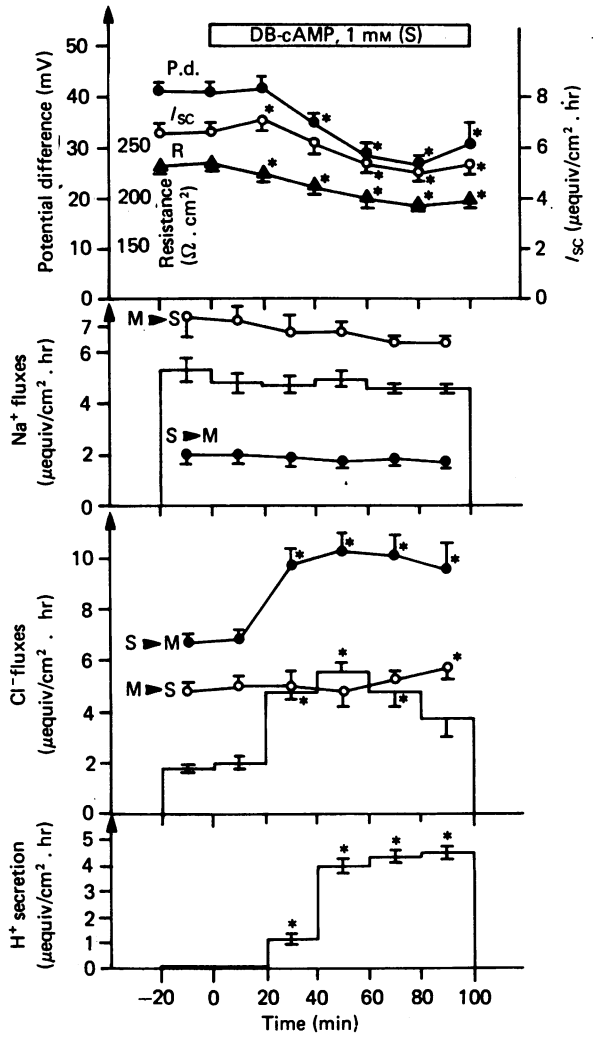

Fig. 2. Effects of DBcAMP on electrical parameters, Na^+ fluxes (n = 4), Cl^- fluxes (n = 5) and H^+ secretion (n = 4) in isolated dog gastric mucosa. All symbols represent the same as in Fig. 1.

1·25 ± 0·75 μequiv/cm².hr; and net flux of Na^+ from mucosal to serosal was 5·09 ± 0·92 μequiv/cm².hr. When 10 mM-cyclic AMP was present in the serosal solution, p.d. and I_{SC} increased significantly at 20 and 40 min; then there was a tendency to return to the control values. Resistance decreased throughout all experiments following the addition of cyclic AMP. Simultaneously, the net flux of Na^+ increased due to the increase in the unidirectional flux of Na^+ from the mucosal to serosal side. The unidirectional flux of Cl^- was increased in both directions. In the later stage, the increase in Cl^- flux from the mucosal to serosal side was greater than the increase from the serosal to mucosal side; this resulted in the decrease in the net flux of Cl^-. No acid secretion was observed after the addition of cyclic AMP.

The protocol for the effects of DBcAMP on isolated dog gastric mucosae was the

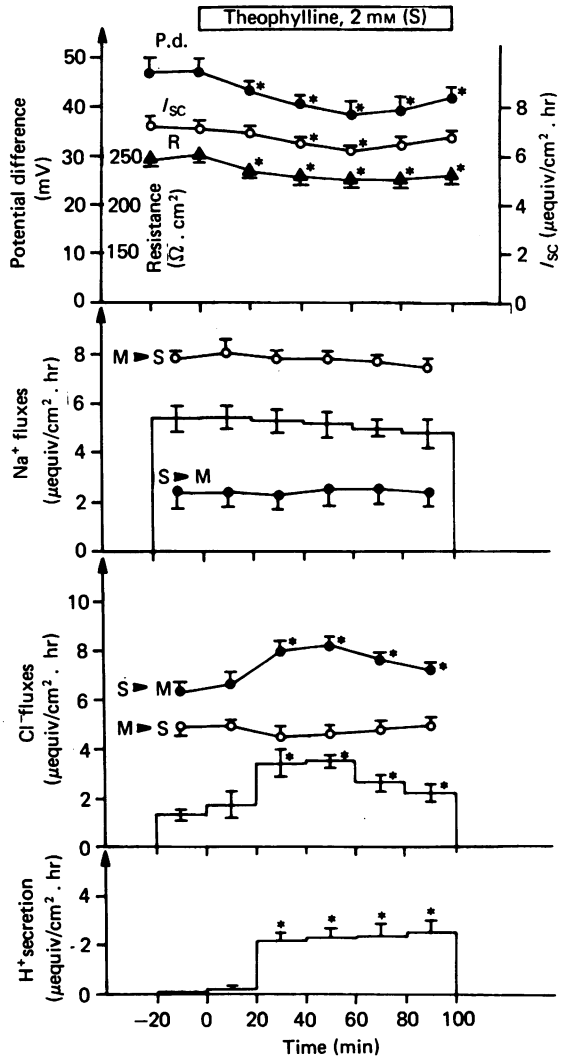

Fig. 3. Effects of theophylline on electrical parameters, Na^+ fluxes (n = 3), Cl^- fluxes (n = 5) and H^+ secretion (n = 4) in isolate dog gastric mucosa. All symbols represent the same as in Fig. 1.

same as for the experiments with cyclic AMP. Similarly, R was decreased after DBcAMP addition into the serosal solution (Fig. 2). However, the I_{SC} increased only in the first 20 min period, then decreased. The p.d. significantly decreased after 40 min. There were no significant changes in Na^+ fluxes in either direction throughout the experiments. The net flux of Cl^- was increased within 40 min after DBcAMP. This increase was due to an increase in the unidirectional flux of Cl^- from the serosal to mucosal side. The unidirectional flux of Cl^- from mucosal to serosal was also increased in the later stage. Acid secretion commenced from 20 to 30 min after the addition of DBcAMP (also see Figs. 4 and 5) and reached a plateau at 30–40 min.

Responses of the isolated dog gastric mucosa to theophylline (present in the

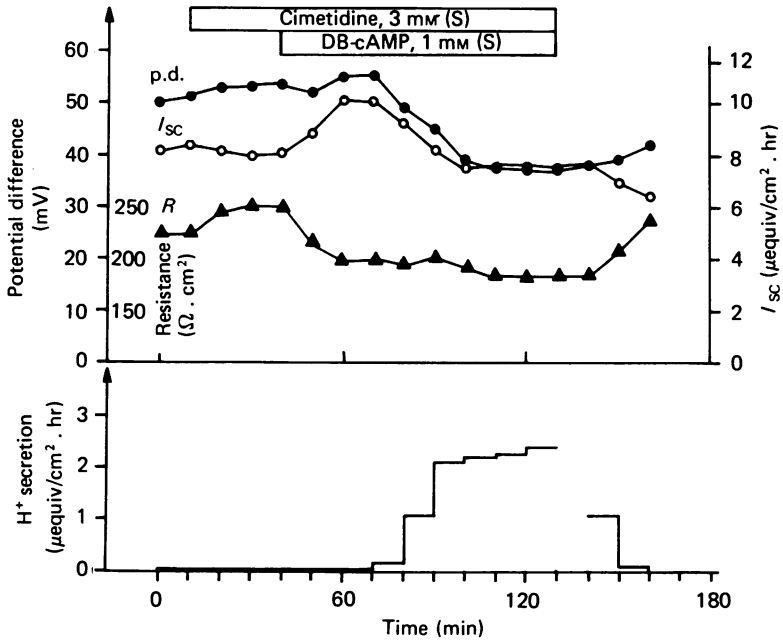

Fig. 4. Effects of DBcAMP on electrical parameters and H^+ secretion in cimetidine-pretreated gastric mucosa.

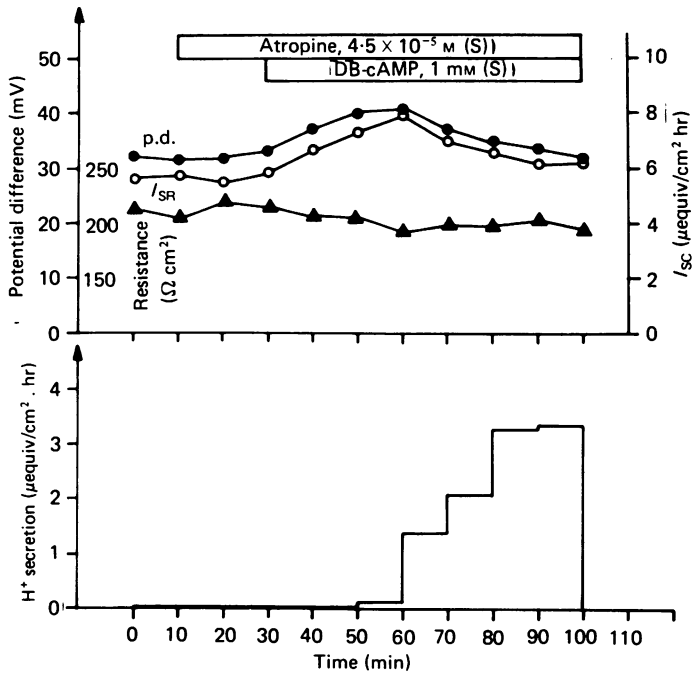

Fig. 5. Effects of DBcAMP on electrical parameters and H^+ secretion in atropine-pretreated gastric mucosa.

serosal solution) were similar to those of DBcAMP, i.e. decreases in p.d., I_{SC} and R, increases in H^+ and Cl^- secretion, and no change in Na^+ transport (Fig. 3). However, the degree of all of these changes was less than those produced by DBcAMP, e.g. maximal H^+ secretion after theophylline was $2{\cdot}52 \pm 0{\cdot}54$ μequiv/cm^2.hr, while maximal H^+ secretion reached $4{\cdot}52 \pm 0{\cdot}25$ μequiv/cm^2.hr after DBcAMP.

Another series of experiments was performed in which either cyclic AMP or DBcAMP was present in the mucosal solution. There were no significant changes in any of the electrical parameters, Na^+ fluxes (Table 1) or Cl^- fluxes (Table 2). No H^+ secretion was observed when DBcAMP was present in the mucosal solution.

TABLE 1. Effects of mucosal cyclic AMP on Na^+ fluxes across isolated dog gastric mucosa

	P.d.	I_{SC}	R	$J^{Na^+}_{S\to M}$	$J^{Na^+}_{M\to S}$	$J^{Na^+}_{Net}$
Control	49 ± 1	$7{\cdot}66 \pm 0{\cdot}24$	238 ± 7	$2{\cdot}49 \pm 0{\cdot}31$	$7{\cdot}82 \pm 0{\cdot}79$	$5{\cdot}33 \pm 0{\cdot}48$
Cyclic AMP	44 ± 3	$6{\cdot}42 \pm 0{\cdot}61$	254 ± 7	$2{\cdot}68 \pm 0{\cdot}38$	$6{\cdot}69 \pm 0{\cdot}37$	$4{\cdot}00 \pm 0{\cdot}32$

All values are in μequiv/cm^2.hr, except p.d. and R which are in mV and Ω.cm^2, respectively. $n = 3$.

TABLE 2. Effects of mucosal dibutyryl cyclic AMP on Cl^- fluxes and H^+ secretion across isolated dog gastric mucosa

	P.d.	I_{SC}	R	$J^{Cl^-}_{S\to M}$	$J^{Cl^-}_{M\to S}$	$J^{Cl^-}_{Net}$	H^+
Control	43 ± 2	$6{\cdot}36 \pm 0{\cdot}40$	252 ± 11	$6{\cdot}44 \pm 0{\cdot}40$	$4{\cdot}43 \pm 0{\cdot}19$	$2{\cdot}01 \pm 0{\cdot}21$	0
DBcAMP (1 mM)	41 ± 1	$6{\cdot}34 \pm 0{\cdot}21$	246 ± 13	$6{\cdot}85 \pm 0{\cdot}46$	$5{\cdot}10 \pm 0{\cdot}32$	$1{\cdot}75 \pm 0{\cdot}32$	0

All values are in μequiv/cm^2.hr, except p.d. and R which are in mV and Ω.cm^2, respectively. $n = 3$.

A typical response of isolated dog gastric mucosa to DBcAMP after pretreatment with cimetidine is shown in Fig. 4. Before the addition of cimetidine, the resting mucosa reached a steady state with no spontaneous H^+ secretion. Following the addition of 3 mM-cimetidine to the serosal solution, p.d. and R increased and I_{SC} slightly decreased. None of these differences were statistically significant. Although the mucosa was pretreated with the H2-blocker, the response of the tissue to DBcAMP was exactly the same as the response without cimetidine (compared to Fig. 2). Washing both cimetidine and DBcAMP from the serosal side abolished H^+ secretion; p.d. and R tended to recover.

Stimulation of isolated dog gastric mucosa by DBcAMP was also not affected by the cholinergic blocker, atropine. A typical response is illustrated in Fig. 5. There was an initial increase in p.d. and I_{SC} after DBcAMP; acid secretion commenced when p.d. and I_{SC} began to decrease.

An additional three experiments were performed in which K^+ fluxes were determined after cyclic AMP administration (Fig. 6). The unidirectional flux of K^+ from mucosal to serosal side was significantly increased in the 20 min period after 10 mM-cyclic AMP, which resulted in an increase in the net flux of K^+ from the mucosal to serosal side.

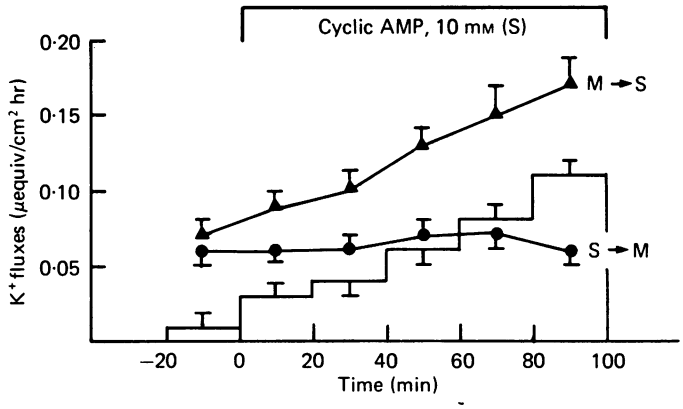

Fig. 6. Effects of cyclic AMP on K^+ fluxes (n = 3) in isolated dog gastric mucosa. All symbols represent the same as in Fig. 1.

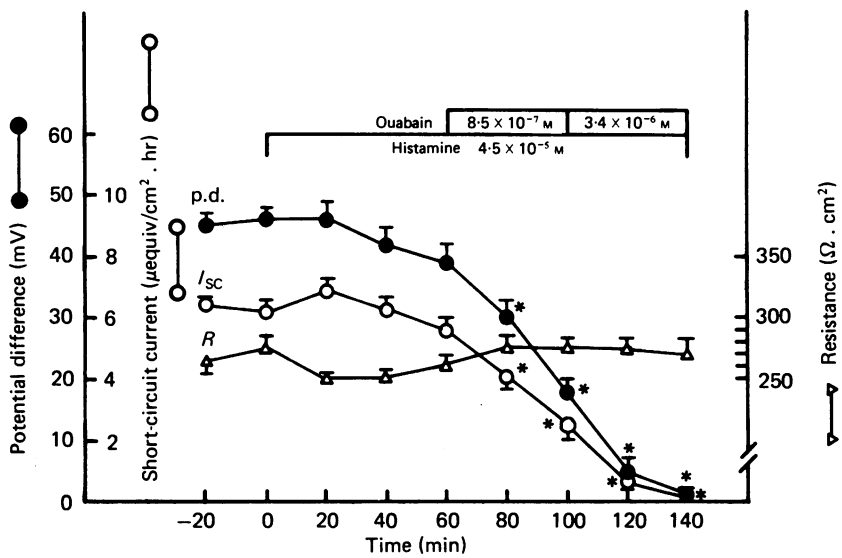

Fig. 7. Effects of ouabain on potential difference, short-circuit current and resistance in isolated dog gastric mucosa. Histamine was added into the serosal solution 60 min before ouabain administration. n = 10.

* Significant difference from values at 40–60 min ($P < 0{\cdot}05$, paired Student t test).

Effects of ouabain

Fig. 7 illustrates the effects of ouabain on p.d., I_{SC} and R in isolated dog gastric mucosae, which had been stimulated by histamine to secrete H^+ and Cl^- for 1 hr. Histamine ($4{\cdot}5 \times 10^{-5}$ M, added into the serosal side) produced a decrease in R, an initial increase in I_{SC}, and no change in p.d. Addition of $8{\cdot}5 \pm 10^{-7}$ M-ouabain into the serosal solution was followed by a progressive decrease in p.d. and I_{SC}, while R

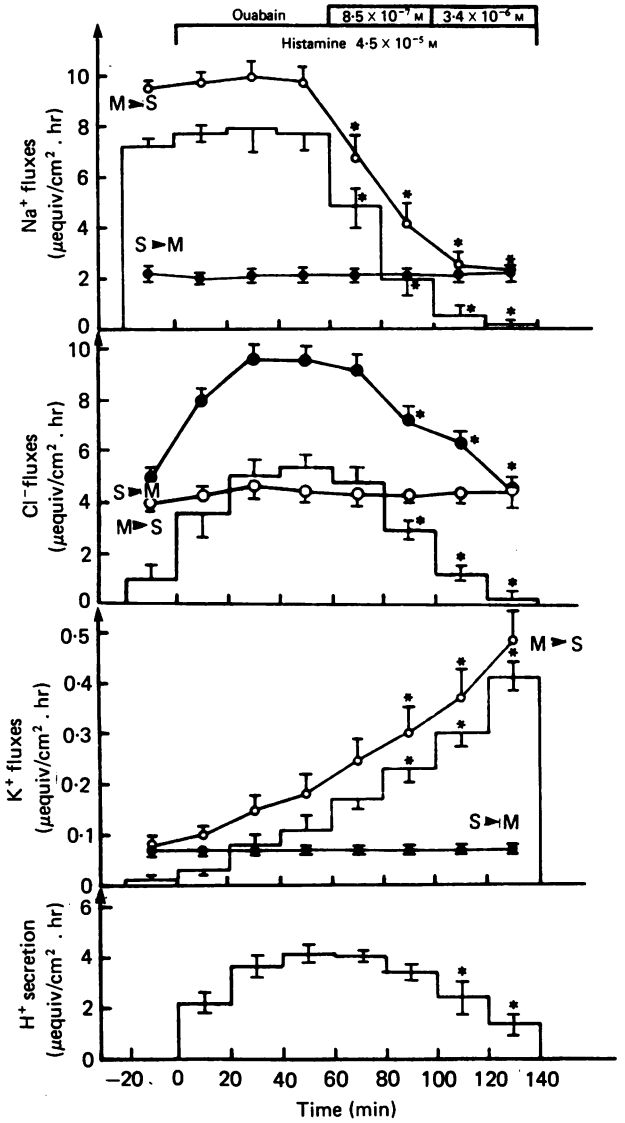

Fig. 8. Effects of ouabain on Na^+ fluxes (n = 3), Cl^- fluxes (n = 3), H^+ secretion (n = 4) and K^+ fluxes (n = 3) in isolated dog gastric mucosa.

* Significant difference from values at 40–60 min ($P < 0.05$, paired Student t test). All other symbols represent the same as in Fig. 1.

remained relatively stable. When the ouabain concentration was increased (fourfold, 3.4×10^{-6} M) in the serosal solution, p.d. and I_{SC} decreased to zero.

Ouabain effects on H^+ secretion, Na^+, Cl^- and K^+ fluxes across isolated dog gastric mucosae are shown in Fig. 8. Unidirectional fluxes of Na^+ from the mucosal to serosal side, and from serosal to mucosal side and net fluxes of Na^+ (from mucosal to serosal side, represented by histogram) remained stable in histamine-stimulated mucosae. Addition of ouabain to the serosal solution of gastric mucosae significantly inhibited

net flux of Na^+ due to the inhibition of the unidirectional mucosal to serosal flux. The inhibitory response was significant as early as 20 min following $8{\cdot}5 \pm 10^{-7}$ M-ouabain addition. Net Na^+ movement was reduced almost to zero at 20 min following $3{\cdot}4 \pm 10^{-6}$ M-ouabain and totally abolished within the next 20 min.

The net flux of Cl^- from the serosal to mucosal side was stimulated by histamine from $0{\cdot}94 \pm 0{\cdot}6$ to $5{\cdot}23 \pm 0{\cdot}58$ μequiv/cm^2.hr. Treatment of the mucosae with ouabain significantly decreased the net flux of Cl^- within 40 min. The net flux of Cl^- was further reduced by $3{\cdot}4 \times 10^{-6}$ M-ouabain and essentially reached zero in the last 20 min period of measurement.

Secretion of H^+ was stimulated from zero to $4{\cdot}12 \pm 0{\cdot}37$ μequiv/cm^2.hr by histamine. Addition of $8{\cdot}5 \times 10^{-7}$ M-ouabain produced a slight but insignificant decrease in H^+ secretion; H^+ secretion then decreased in $3{\cdot}4 \pm 10^{-6}$ M-ouabain and was significantly reduced but not abolished in the last 20 min period of measurement.

The unidirectional flux of K^+ from the mucosal to serosal side was increased in $4{\cdot}5 \times 10^{-5}$ M-histamine and was further increased by ouabain. In order to determine whether the increase in the unidirectional flux of K^+ from mucosal to serosal side was the effect of ouabain or due to the long term effect of histamine, K^+ flux (mucosal to serosal side) was measured with histamine or ouabain treatment alone. Both histamine and ouabain increased the unidirectional flux of K^+ from the mucosal to serosal side by 107 and 164 %, respectively, while ouabain potentiated the histamine effect by 497 %.

Effects of furosemide

When 10^{-4} M-furosemide was present in the serosal solution, p.d. and I_{SC} decreased while resistance increased. This correlated with the decrease in the unidirectional flux of Na^+ from the mucosal to serosal side and the decrease in the net flux of Na^+. Neither the unidirectional flux of Na^+ from serosal to the mucosal side, nor Cl^- fluxes in either direction was changed after furosemide (Fig. 9). When the concentration of furosemide was increased to 10^{-3} M, decreases in the unidirectional flux of Cl^- from the serosal to mucosal side and the net flux of Cl^- were also observed. However, 10^{-3} M-furosemide produced no effects on K^+ fluxes and histamine-stimulated H^+ and Cl^- secretions.

DISCUSSION

Cyclic AMP stimulated the net flux of Na^+ from the mucosal to serosal side when present in the serosal solution of isolated dog gastric mucosa. Similar effects have been reported from frog skin (Hall, O'Donoghue, O'Regan & Penny, 1976) and toad urinary bladder (Mendoza, Handler & Orloff, 1970). The increase in the unidirectional flux of Na^+ from the mucosal to serosal side could be due to either to an increase in the permeability of the serosal membrane to Na^+ or an increase in the rate of the Na^+ pump or both. Cyclic AMP also increased the unidirectional flux from the mucosal to serosal side for Cl^- and K^+, which suggests that an increase in the permeability of the serosal membrane is a distinct possibility. However, an increase in the rate of the Na^+ pump cannot be excluded.

Since cyclic AMP failed to stimulate H^+ secretion, the effects of DBcAMP, a

derivative of cyclic AMP to which the membrane is more permeable, were examined. The effects of DBcAMP on the gastric mucosa were very similar to the effects of histamine (Kuo & Shanbour, 1979): stimulation of H^+ and Cl^- secretion; decrease in p.d. and R; and no effect on Na^+ transport. The only exception was that there was a time lag of 20–30 min for DBcAMP to initiate acid secretion. Butyric acid (2 mM),

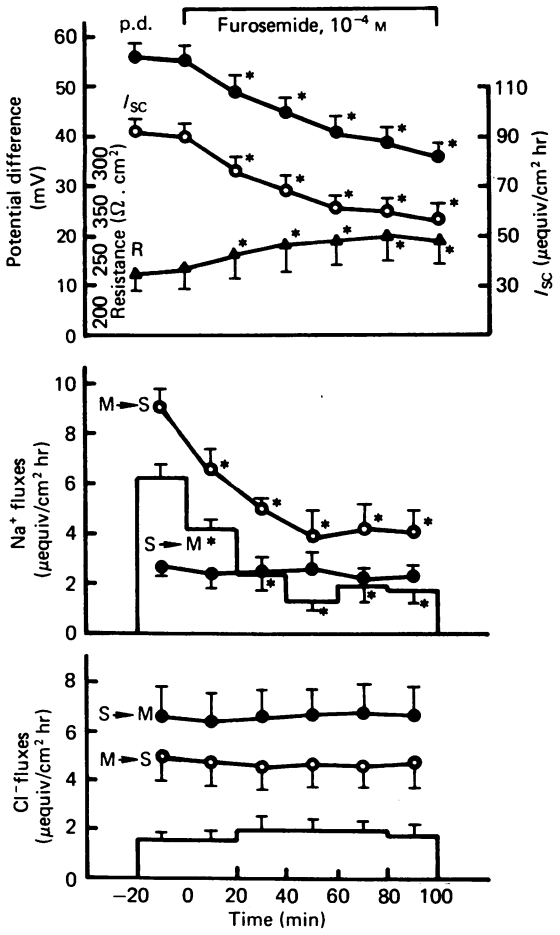

Fig. 9. Effects of furosemide on electrical parameters, Na^+ fluxes (n = 3) and Cl^- fluxes (n = 3) in isolated dog gastric mucosa. All symbols represent the same as in Fig. 1.

or DBcGMP (1 mM) produced no effect on gastric transport, providing further evidence that the effects of DBcAMP are due to an increase in intracellular cyclic AMP. Theophylline, an inhibitor of phosphodiesterase that results in an increase in intracellular cyclic AMP, had similar but lesser effects than DBcAMP. Stimulation of H^+ secretion by DBcAMP has also been reported for frog (Shoemaker, Buckner, Spenney & Sachs, 1974), necturus (Spenney, Strych, Nakajima, Hirschowitz & Sachs, 1972), rat (Brennan, Arkbakov, Stefankiewicz & Groves, 1975) and rabbit (Fromm, Schwartz & Ouijano, 1975) gastric mucosae *in vitro*, and for rat stomach

in vivo (Jawaharlal & Berti, 1972). However, DBcAMP did not initiate acid secretion in dog stomach *in vivo* (Mao, Shanbour, Hodgins & Jacobson, 1972). The discrepancies for the present study are obviously due to differences in preparation. Nevertheless, the isolated gastric mucosal preparation provides more direct information concerning the action of DBcAMP on acid secretion, since DBcAMP may be metabolized before reaching parietal cells when applied either intra-arterially or intravenously *in vivo*.

Ouabain is known to inhibit cation transport and the activity of Na^+- K^+-dependent ATPase in most tissues (Skou, 1965). In oxygenated *in vitro* frog gastric mucosa, Davenport & Chavre (1952) reported that ouabain inhibited the active transport of H^+. Sernka & Hogben (1969) observed inhibition of H^+ and Cl^- transport with ouabain, while the net flux of Na^+ and membrane R remained unaffected in rat gastric mucosa. In isolated sheep rumen epithelium, Harrison, Keynes, Rankin & Zurich (1975) found inhibition of net fluxes of Na^+, Cl^- and K^+ by ouabain. The concentrations of ouabain in the above experiments ranged from 10^{-5} to 10^{-3} M. With a relatively lower concentration of ouabain, Flemström & Öbrink (1972) observed that 10^{-7} M-ouabain inhibited only the active transport of Na^+ during a slightly hypoxic state of isolated frog gastric mucosa, while Cl^- and H^+ transport were not affected. In the canine gastric flap preparation *in vivo*, Kuo, Bowen, Smith, Sernka, Jacobson & Shanbour (1974) found that ouabain (50 μg/kg with intravenous bolus) decreased the p.d. of histamine-stimulated gastric mucosae, but that the rate of acid secretion was unaltered. Similar results were observed with ouabain 25 μg/kg, which is the usual veterinary dose of digitalis for dogs, and dogs do not survive when the ouabain concentration is increased to 100 μg/kg (unpublished observations). The concentrations of ouabain used in the present *in vitro* experiments, $8{\cdot}5 \times 10^{-7}$ and $3{\cdot}4 \times 10^{-6}$ M, correspond to 25 and 100 μg/kg *in vivo*, respectively. The decrease in gastric p.d. produced by ouabain with these two concentrations is essentially the same in both *in vivo* and *in vitro* studies. Thus ouabain, in the doses of veterinary or medical usage (0·1–0·5 mg, intravenously), is ineffective on H^+ secretion, but it does decrease the p.d. and specifically inhibits Na^+ transport. Increasing the concentration of ouabain to $3{\cdot}4 \times 10^{-6}$ M or higher *in vitro*, which corresponds to the lethal dose *in vivo*, may result in inhibition of H^+ secretion, but this effect has no physiological significance. Furthermore, with higher concentrations of ouabain in the *in vitro* studies, there was usually a steady decrease in the I_{SC} to zero, and a decrease in R. The effect of ouabain was irreversible, indicating a general toxic effect of ouabain on tissues. However, all of the above responses were not present when mucosae were treated with $8{\cdot}5 \times 10^{-7}$ M-ouabain.

The primary action of ouabain is probably inhibition of the Na^+–K^+–ATPase located on the serosal membrane. This would account for the early effect of inhibition of active Na^+ transport into the serosal solution. The resultant increase in intracellular Na^+ would retard Cl^- extrusion into the mucosal solution, resulting in a secondary effect on Cl^- transport. The decreased Cl^- transport would then produce a tertiary effect of decreased H^+ secretion (Kuo & Shanbour, 1979). The increased K^+ transport induced by ouabain probably reflects the inhibition of the Na^+–K^+ pump and the selective permeability of the serosal membrane, resulting in passive diffusion of K^+ from the higher intracellular compartment into the serosal solution.

Furosemide is a potent diuretic that in renal tubules may produce its effects by

reducing Na^+ reabsorption (Buchborn & Anastasakis, 1964). In flounder intestine, Frizzell, Smith, Vosburgh & Field (1979) found that the addition of furosemide to the mucosal solution inhibited I_{SC} and reduced unidirectional influxes of Na^+ and Cl^- from the mucosal solution suggesting that the coupled influx mechanism mediates a one-for-one entry of these ions into the cell from the mucosal solution. With 10^{-4} M-furosemide in the serosal solution in isolated dog gastric mucosa, the unidirectional

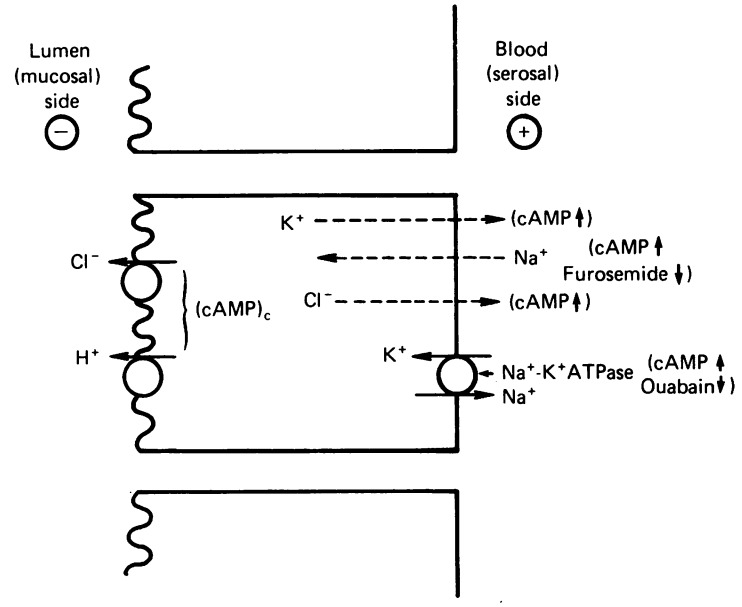

Fig. 10. Schematic model of dog gastric mucosa. Continuous lines represent active transport processes and dashed lines represent passive processes. $(cAMP)_c$ represents intracellular cyclic AMP. ↑ and ↓ represent stimulation and inhibition, respectively.

flux of Na^+ from the mucosal to serosal side was inhibited simultaneously with the decrease in p.d. and I_{SC}, and the increase in R. However, Cl^- transport was not affected. Raising the furosemide concentration to 10^{-3} M slightly inhibited the net flux of Cl^- from the serosal to mucosal side. The selective action of furosemide on Na^+ flux indicates that Na^+ and Cl^- move via separate transport pathways across the serosal border in dog gastric mucosa.

These observations suggest a model for ion transport in the dog gastric mucosa (Fig. 10).

(*a*) At the basolateral membrane, Na^+ moves in and K^+ out of the cell down their electrochemical gradients. This uptake of Na^+ may be inhibited by furosemide. The Na^+ carried into the cell is then extruded by the Na^+–K^+ exchange pump, which has been identified in the basolateral membranes of a wide variety of epithelial cells (Mills & DiBona, 1978; Stirling, 1972; Klyce & Wong, 1977). This pump mechanism is energy-dependent, inhibited by ouabain, and may be stimulated by cyclic AMP. Cyclic AMP also increases the permeability of the basolateral membrane for the outflux of K^+ and Cl^- into the serosal solution and the influx of Na^+ into the cell, which provides more intracellular Na^+ to pump out through the membrane.

(*b*) At the luminal side, H^+ is secreted against its electrochemical gradient. Secretion is stimulated by secretagogues (Kuo & Shanbour, 1978) and by an increase in intracellular cyclic AMP. Acid secretion is dependent upon Na^+ and K^+ in the serosal solution, and is also dependent upon the active transport of Cl^- (Kuo & Shanbour, 1979). Although Cl^- and H^+ secretion are closely related, the secretion cannot be in the HCl form, since Cl^- transport was completely abolished by ouabain treatment whereas H^+ secretion did not show the same pattern.

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